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Nutlet micromorphological characters of *Teucrium* taxa (Lamiaceae) in Libya

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Abstract

The nutlet micromorphology of 11 *Teucrium* taxa from Libya was examined using scanning electron microscopy (SEM). The variation in shape, color, size and ornamentation of mericarp helped for taxa discrimination. Furthermore, *T. capitatum* and *T. polium* subsp. *flavovirens* are reported here for the first time in Libya.

Key words: micromorphology, nutlet surface, scanning electron microscopy, subfamily Ajugoideae, tribe Teucrieae

Introduction

Teucrium Linnaeus (1753: 562) is a complex and problematic genus with more than 300 species with of a ubiquitous distribution around the world, particularly in the Mediterranean basin (Radulovic *et al.* 2012). Consequently, numerous taxonomic studies were performed concerning this genus (Servettaz *et al.*1992, Grubešić *et al*. 2007, Sánchez-Gómez *et al*. 2013).

For both generic and specific demarcation, the nutlet micromorphology of the genus is accepted as the most valuable taxonomic characters (Navarro & EL Oualidi 1999, Dinç *et al.* 2008, Genç *et al.* 2015). In the intervening time, the nutlet characters have been shown to be phylogenetically informative (Marin *et al.* 1994). The presence or absence of trichomes, their typology and both the presence and the density of oil glands served as an additional marker at specific and subspecific level (Navarro 1995, Navarro & EL Oualidi 2000, Eshratifar *et al*. 2011).

Teucrium is a controversial genus in Libya and was represented by 13 species (Siddiqi 1985); *T. apollinis* Maire & Weiller (1939: 86), *T. barbeyanum* Asch. & Taub. ex E.A.Durand & Barratte (1910: 191), *T. brevifolium* Schreber (1774: 27), *T. campanulatum* Linnaeus (1753: 562), *T. compactum* Clem. ex Lagasca y Segura (1816: 17), *T. davaeanum* Cosson (1889: 102), *T. divaricatum* Sieber ex Heldreich (1857: 290), *T. flavum* Linnaeus (1753: 565), *T. fruticans* Linnaeus (1753: 562), *T. lini-vaccarii* Pampanini (1914: 17), *T. pilosum* (Decaisne 1834: 250) Ascherson & Schweinfurth (1887: 189), *T. polium* Linnaeus (1753: 566) and *T. zanonii* Pampanini (1917: 153). Among these taxa 5 are endemic in Libya; *T. apollinis, T. barbeyanum, T. davaeanum, T. lini-vaccari* and *T. zanonii.*

El-Tajory (2004) reported 13 taxa in Libya with the addition of *T. alpestre* Sm. in Sibthorp & Smith (1809: 195) subsp. *alpestre*, *T. alpestre* subsp. *gracile* (Barbey & Fors.-Major 1972: 271) Wood (1972: 261) and *T. microphyllum* Desfontaines (1807: 300) and the exclusions of *T. barbeyanum*, *T. campanulatum* and *T. flavum*.

Therefore, this study is focused on the documentation of *Teucrium* taxa distributed in Libya, as well as the micromorphological description of their nutlets with the aim of contributing to its taxonomy. Two new taxa, *T. capitatum* and *T. polium* subsp. *flavovirens*, are reported in this work.

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TABLE 1. Collection data of *Teucrium* taxa studied ("*" endemic taxa of Libya). The voucher specimens were kept in the herbaria at Faculty of Science of Alexandria University (ALEX), and Faculty of Science of Omar EL-Mukhtar University.

Voucher number	Section	Taxa	Locatity (datum WGS84)
1–3	T. sect. Chamaedrys	*Teucrium barbeyanum*[*]	Shahhat Susah Coordinate pair: N32°30.0756 E21°31.8756 Latitude N32°30.0756 Longitude E21°31.8756
4–6	T. sect. Polium	*Teucrium apollinis*[*]	Shahhat Susah Coordinate pair: N32° 50°30.42 E21° 51°7.2 Latitude N32° 50°30.42 Longitude E21° 51°7.2
7–8		*Teucrium capitatum*	Tarhonah Coordinate pair: N32° 29°48.78 E13° 37°37.08 Latitude N32° 29°48.78 Longitude E13° 37°37.08
9–11		*Teucrium davaeanum*[*]	Wadi El Quttarh Coordinate pair: N32° 01°35.82 E20° 24°45.48 Latitude N32° 01°35.82 Longitude E20° 24°45.48
12–14		*Teucrium lini-vaccarii*[*]	Quasser–El Quaar Coordinate pair: N32° 35°20.28 E13° 50°18.18 Latitude N32° 35°20.28 Longitude E13° 50°18.18
15–17		*Teucrium polium*	Sirut Coordinate pair: N31° 08°56.1 E16° 34°35.16 Latitude N31° 08°56.1 Longitude E16° 34°35.16
18–19		*Teucrium polium* subsp. *flavovirens*	Al Hameida escarpment Coordinate pair: N32° 24°52.98 E20° 32°17.88 Latitude N32° 24°52.98 Longitude E20° 32°17.88
20–22		*Teucrium zanonii*[*]	Dryannah Coordinate pair: N32° 19°42.12 E20° 16°34.86 Latitude N32° 19°42.12 Longitude E20° 16°34.86
23–25	T. sect. Teucrium	*Teucrium brevifolium*	Lathroun–Ras El Hellal Coordinate pair: N32° 52 305'°0 E22° 15°6.12 Latitude N32° 52 305'°0 Longitude E22° 15°6.12
26–28		*Teucrium campanulatum*	Wadi Errieg Coordinate pair: N32° 32 230°0 E20° 42°56.82 Latitude N32° 32 230°0 Longitude E20° 42°56.82
29–30		*Teucrium fruticans*	El Rabtta–El Assbeh Coordinate pair: N32° 07°12.6 E21° 52°16.14 Latitude N32° 07°12.6 Longitude E21° 52°16.14

TABLE 2. Nutlet micromorphological characters for the studied taxa of *Teucrium*.

Section	Taxa	Nutlet Color	Nutlet length (mm)	Nutlet width (mm)	Nutlet length / Nutlet width	Nutlet shape	Nutlet surface	Thickness of muri (µm)	Diameter of lacuna (µm)	Length of abscission scar (L. mm)	Width of abscission scar (W. mm)	L/W of abscission Scar	Shape of the abscission scar	Length of abscission scar / length of Nutlet (%)
T. sect. Chamaedrys	*T.barbeyanum*	Dark brown	1.02	0.54	1.88	Elliptic	Hairy	-	-	0.43	0.39	1.10	Ellipitic	46
T. sect. Polium	*T.apollinis*	Dark brown	0.77	0.53	1.45	Elliptic	Glabrous	3.10	6.90	0.42	0.40	1.05	Elliptic	43
	T.capitatum	Dark brown	1.04	0.66	1.60	Elliptic	Glabrous	-	-	0.55	0.60	0.91	Orbicular	55
	T.davaeanum	Dark brown	0.61	0.45	1.35	Elliptic	Glabrous	2.30	10.80	0.35	0.35	1.00	Ellipitic	55
	T.lini-vaccarii	Light brown	0.61	0.40	1.52	Elliptic	Glabrous	2.20	10.30	0.38	0.35	1.08	Ellipitic	60
	T.polium	Dark brown	0.83	0.52	1.59	Elliptic	Glabrous	2.50	10.00	0.52	0.44	1.18	Ellipitic	63
	T.polium subsp. *flavovirens*	Dark brown	0.96	0.58	1.65	Elliptic	Glabrous	2.50	9.40	0.47	0.31	1.51	Elliptic	51
	T.zanonii	Dark brown	0.85	0.60	1.41	Elliptic	Glabrous	1.67	11.67	0.48	0.43	1.11	Ellipitic	56
T. sect. Teucrium	*T.brevifolium*	Light brown	1.46	0.8	1.82	Elliptic	Hairy	-	-	0.97	0.88	1.10	Ellipitic	62
	T.campanulatum	Brown	1.30	0.77	1.68	Elliptic	Hairy	-	-	0.86	0.71	1.21	Ellipitic	68
	T.fruticans	Light brown	1.43	0.61	2.34	Oblong	Hairy	-	-	1.22	0.96	1.27	Ellipitic	74

Materials & Methods

Specimens of 11 *Teucrium* taxa were collected through two flowering seasons (2009–2010) from 12 different localities in Libya (Table 1). The specimens were identified according to Siddiqi (1985) and by comparison with the specimens kept in the herbarium of Omar EL-Mukhtar University (El-Bayda, Libya) and in the Libyan collection of Loutfy Boulos housed in Alexandria University (Alexandria, Egypt). Furthermore, some specimens were kindly identified by Teresa Navarro (Málaga University, Spain). The voucher specimens were kept in the herbaria at Faculty of Science of Alexandria University (ALEX), and Faculty of Science of Omar EL-Mukhtar University.

The nutlets were mounted directly on stubs using double-sided adhesive tape and coated with gold in a polaron JEC-1100E coating unit, then photographed with JEOL JSM-5300 scanning electron microscope (SEM) at Faculty of Science of Alexandria University. The adopted terminology follows Navarro & El Oualidi (2000).

Results

Nutlet description of 11 *Teucrium* taxa from Libya are reported in detail.

Teucrium sect. **Chamaedrys** (Mill.) Schreb.

Teucrium barbeyanum Asch. & Taub. ex E.J. Durand & Barratte
The nutlet is dark brown and elliptic (1.02 × 0.54 mm) with a surface moderately covered with non-glandular hairs decreasing towards the abscission scar. Two types of hairs are recognized; the first is short, non-articulate, erect, conical and smooth-surfaced are observed, whereas, in the second type, they are characterized by long, articulate with smooth basal and micro-papillate apical cell. Smooth-surfaced oil glands exist alongside with the hairs, increasing in number towards the abscission scar. The nutlet sculpture is more or less reticulate, however towards the abscission scar; the surface is rugate to ruminate. The abscission scar is elliptic and occupies about 46% of the nutlet length (Table 2 and Fig. 1, 1–5).

Teucrium sect. **Polium** (Mill.) Schreb.

Teucrium apollinis Maire & Weiller
The nutlet is dark brown and elliptic (0.77 × 0.53 mm), while the surface is glabrous with deep and uniformly distributed areoles. The nutlet sculpture is reticulate of isodiametric cells with incomplete walls especially those within the areoles, however the cells exhibit raised anticlinal and concave external periclinal walls. The thickness of muri is 3.1 µm and the diameter of lacuna is 6.9 µm. The abscission scar is elliptic and occupies about 43% of the nutlet length (Table 2 and Fig. 1, 6–8).

Teucrium capitatum L.
The nutlet is dark brown and elliptic (1.04 × 0.66 mm), while the surface is glabrous with deep areoles becoming shallower towards the abscission scar. The nutlet sculpture is reticulate of isodiametric cells with depressed anticlinal and convex external periclinal walls. The abscission scar is orbicular and occupies about 55% of the nutlet length (Table 2 and Fig. 1, 9–11).

Teucrium davaeanum Coss.
The nutlet is dark brown and elliptic (0.61 × 0.45 mm), while the surface is glabrous with shallow areoles becoming shallower towards the abscission scar. The nutlet sculpture is reticulate of isodiametric cells with raised, wavy anticlinal and concave external periclinal walls. The thickness of muri is 2.3 µm and the diameter of lacuna is 10.8 µm. The abscission scar is elliptic and occupies about 55% of the nutlet length (Table 2 and Fig. 1, 12–14).

Teucrium lini-vaccarii Pamp.
The nutlet is light brown and elliptic (0.61 × 0.40 mm), with a glabrous surface of shallow areoles, shallower towards the abscission scar. The nutlet sculpture is reticulate of isodiametric cells with raised, wavy anticlinal and concave external periclinal walls. The thickness of muri is 2.2 µm and the diameter of lacuna is 10.3 µm. The abscission scar is elliptic and occupies about 60% from the nutlet length (Table 2 and Fig. 1, 15–17).

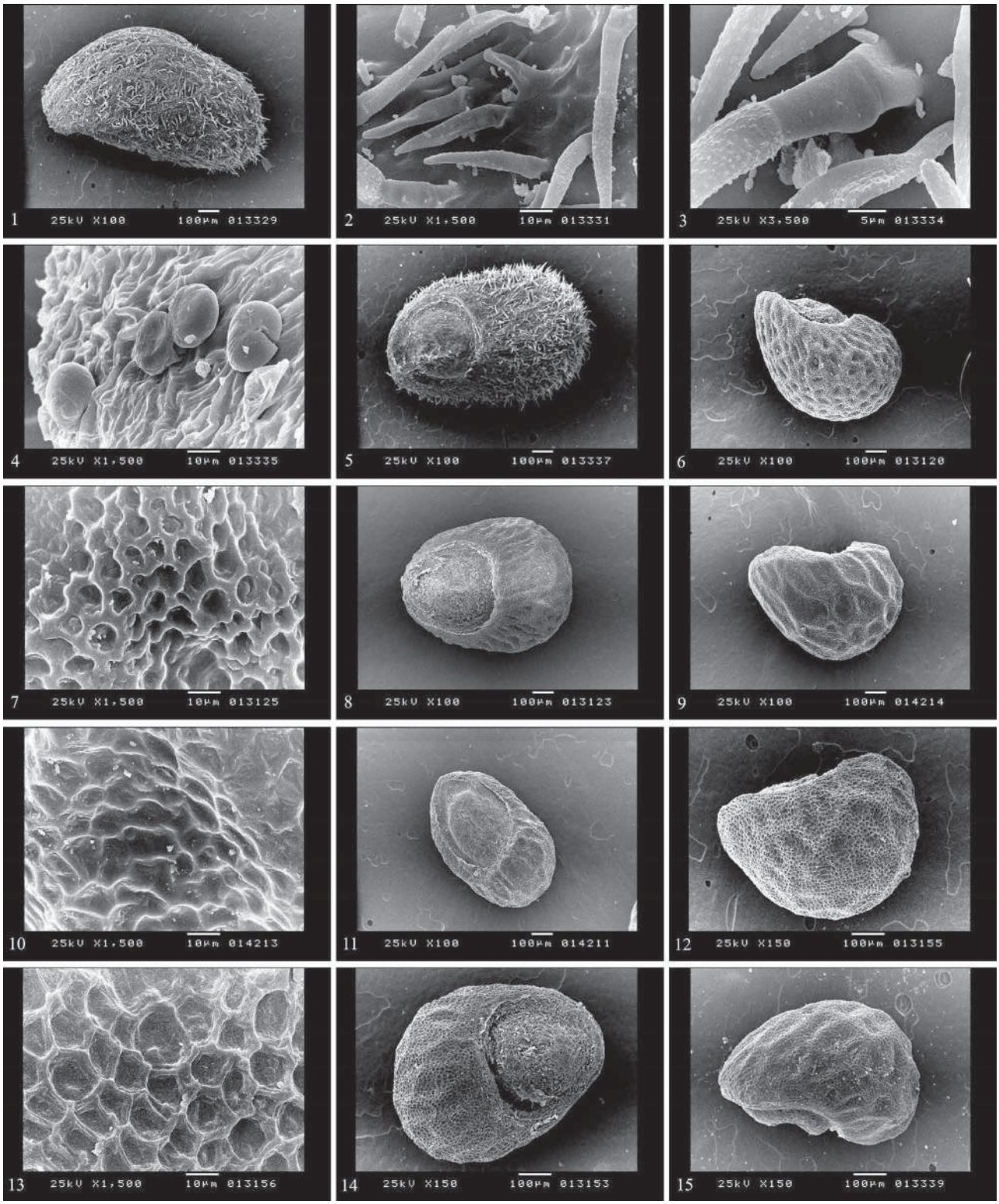

FIGURE 1. *Teucrium* nutlets micrographs using SEM. *T. barbeyanum*, 1–5; *T. apollinis*, 6–8; *T. capitatum*, 9–11; *T. davaeanum*, 12–14; *T. lini–vaccarii*, 15–17; *T. polium*, 18–20; *T. polium* subsp. *flavovirens*, 21–23; *T. zanonii*, 24–26; *T. brevifolium*, 27–31; *T. campanulatum*, 32–36; *T. fruticans*, 37–42.

Teucrium polium L.

The nutlet is dark brown and elliptic (0.83 × 0.52 mm) of glabrous surface with shallow areoles, shallower towards the abscission scar. The nutlet sculpture is reticulate of isodiametric cells with raised, wavy anticlinal and concave external periclinal walls. The thickness of muri is 2.5 µm and the diameter of lacuna is 10µ m. The abscission scar is elliptic and occupies about 63% from the nutlet length (Table 2 and Fig. 1, 18–20).

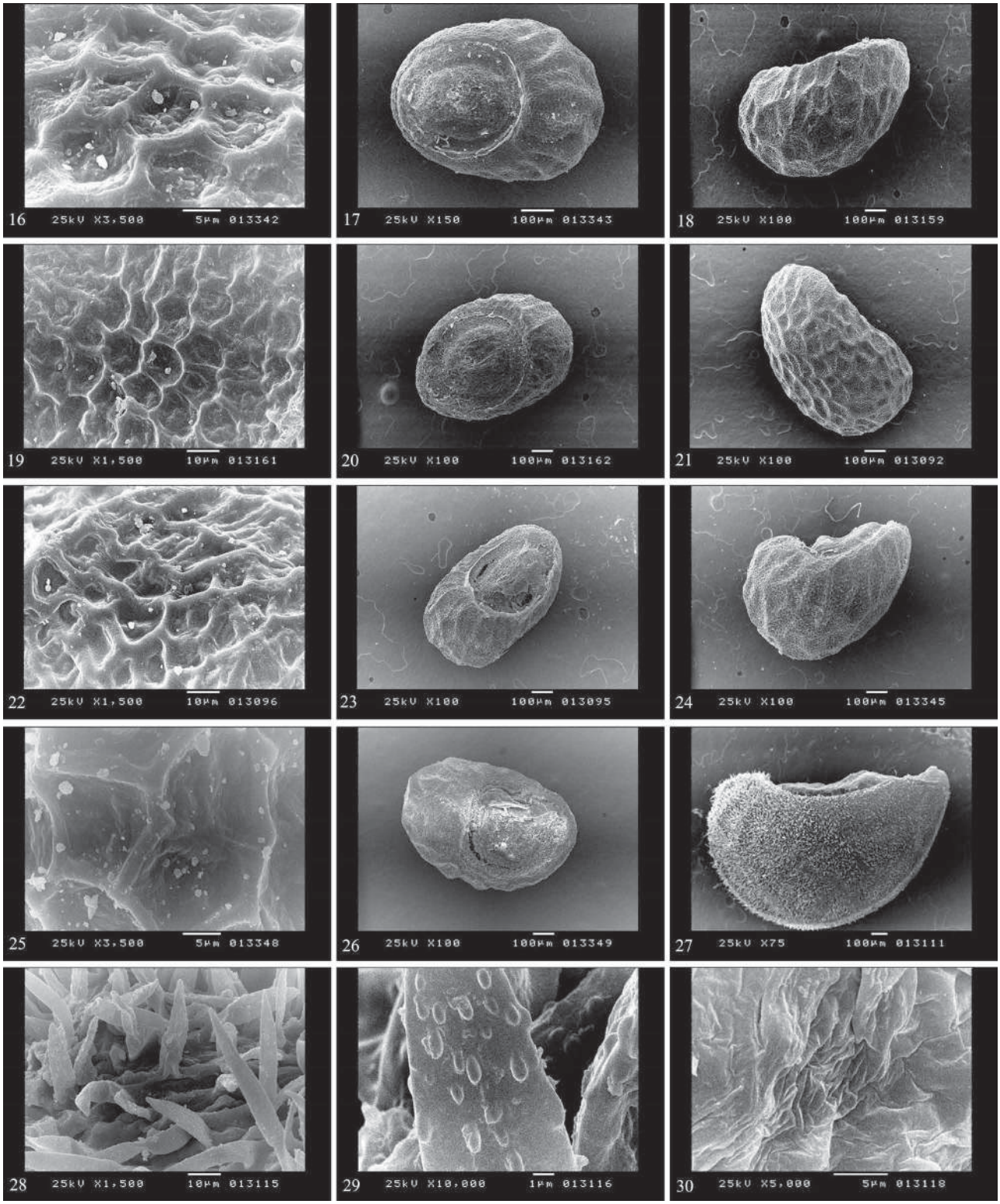

FIGURE 1. **(Continued)** *Teucrium* nutlets micrographs using SEM. *T. barbeyanum*, 1–5; *T. apollinis*, 6–8; *T. capitatum*, 9–11; *T. davaeanum*, 12–14; *T. lini–vaccarii*, 15–17; *T. polium*, 18–20; *T. polium* subsp. *flavovirens*, 21–23; *T. zanonii*, 24–26; *T. brevifolium*, 27–31; *T. campanulatum*, 32–36; *T. fruticans*, 37–42.

Teucrium polium subsp. ***flavovirens*** Batt.

The nutlet is dark brown and elliptic (0.96 × 0.58 mm) of glabrous surface with deep areoles that become shallower towards the abscission scar. The nutlet sculpture is reticulate of isodiametric cells with raised, wavy anticlinal and concave external periclinal walls. The thickness of muri is 2.5 µm and the diameter of lacuna is 9.4 µm. The abscission scar is elliptic and occupies about 51% from the nutlet length (Table 2 and Fig. 1, 21–23).

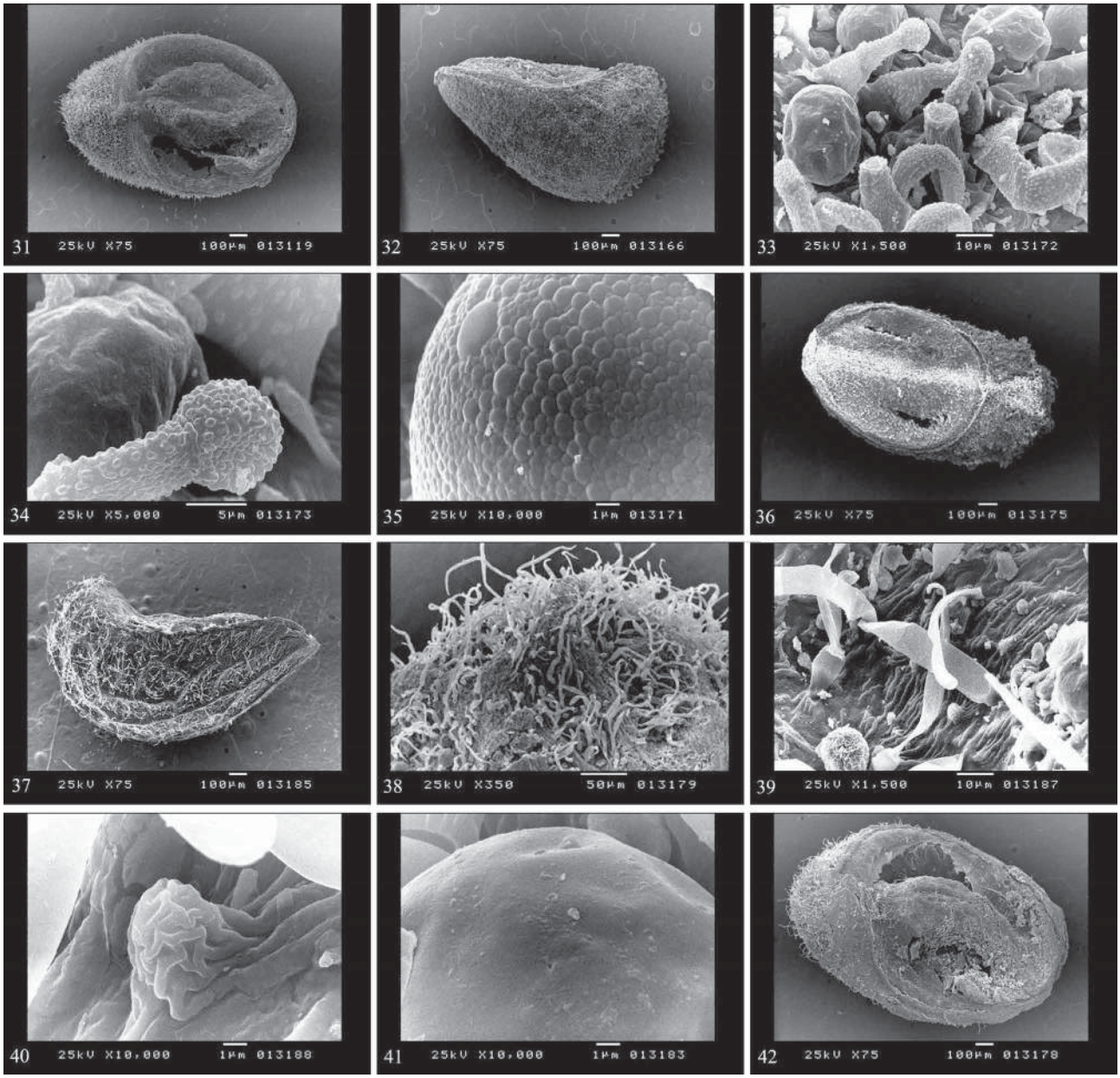

FIGURE 1. **(Continued)** *Teucrium* nutlets micrographs using SEM. *T. barbeyanum*, 1–5; *T. apollinis*, 6–8; *T. capitatum*, 9–11; *T. davaeanum*, 12–14; *T. lini–vaccarii*, 15–17; *T. polium*, 18–20; *T. polium* subsp. *flavovirens*, 21–23; *T. zanonii*, 24–26; *T. brevifolium*, 27–31; *T. campanulatum*, 32–36; *T. fruticans*, 37–42.

Teucrium zanonii Pamp.

The nutlet is dark brown and elliptic (0.85 × 0.60 mm) of glabrous surface with shallow areoles, shallower towards the abscission scar. The nutlet sculpture is reticulate of isodiametric cells with raised, wavy anticlinal and concave external periclinal walls. The thickness of muri is 1.67 µm and the diameter of lacuna is 11.67 µm. The abscission scar is elliptic and occupies about 56% from the nutlet length (Table2, Fig. 1, 24–26).

Teucrium L. sect. **Teucrium**

Teucrium brevifolium Schreb.

The nutlet is light brown and elliptic (1.46 × 0.8 mm) of surface densely covered with non-glandular hairs, decreasing towards the abscission scar. The hairs attain two types; a short, non-articulate, conical or slightly falcate, and micro-papillate and a long, articulate, with smooth basal and micro-papillate apical cell. The nutlet sculpture is striate to ruminate. The abscission scar is elliptic and occupies about 62% from the nutlet length (Table 2 and Fig. 1, 27–31).

Teucrium campanulatum L.

A brown and elliptic nutlet (1.30 × 0.77 mm) of surface moderately covered with glandular hairs changing to glabrous towards the abscission scar. The hairs are long or short, articulate, micro-papillate, with one-celled head. Reticulate-surfaced oil glands are uniformly distributed with dense manner along the nutlet surface. The nutlet sculpture is obscure due to the coverage of hairs and oil glands. The Abscission scar is elliptic and occupies about 68% from the nutlet length (Table 2 and Fig. 1, 32–36).

Teucrium fruticans L.

A light brown and oblong nutlet (1.43 × 0.61 mm) of surface densely covered with non-glandular hairs altering to moderately hairy towards the abscission scar. Hairs are long, articulate and smooth-surfaced. Smooth-surfaced oil glands are uniformly distributed in a moderate manner along the nutlet surface. The nutlet sculpture is striate with distinct protrusions of rugate ornamentation. The abscission scar is elliptic and occupies about 74% from the nutlet length (Table 2 and Fig. 1, 37–42).

In all examined species, the nutlet shape is elliptic, oblong in only *T. fruticans*. The most frequent nutlet color is the dark brown, beside the light brown in *T. brevifolium*, *T. fruticans* and *T. lini-vaccarii*, and the brown in *T. campanulatum*. The nutlets are characterized by conspicuous abscission scar of orbicular shape in *T. capitatum*, and elliptic shape in other species. This scar occupies less than 50% from the nutlet length in both *T. apollinis* and *T. barbeyanum*, more than 60% in *T. brevifolium*, *T. campanulatum*, *T. fruticans* and *T. polium*, and from 50 to 60% in the other species.

Discussion

The present study provides a better insight on the nutlet morphology of *Teucrium* taxa in Libya. According to Marin *et al*. (1994) and Eshratifar *et al*. (2011), the nutlet shape, ornamentation and indumentum type appear to be stable characters, which reveal high systematic value and strongly support the infrageneric classification of the genus.

According to the indumentum type, the nutlet is classified into two groups, hairy in two sections Chamaedrys (*T. barbeyanum*) and Teucrium (*T. brevifolium*, *T. campanulatum* and *T. fruticans*) and glabrous in section Polium (*T. apollinis*, *T. capitatum*, *T. davaeanum*, *T. lini-vaccarii*, *T. polium* and *T. zanonii*). The hairy indumentum considers as a primitive character and thought to be of phylogenetic significance in *Teucrium* (Marin *et al*. 1994). It is notable that the hairiness character is correlated with the relatively large nutlet (more than 1 mm in length). According to Eshratifar *et al*. (2011), the hairs display an ecological role in nutlet dispersal.

In the haired species, *T. campanulatum* is characterized by the presence of glandular hairs alongside the non-glandular ones. Meanwhile, the non-glandular hairs are micropapillate (*T. brevifolium* and *T. campanulatum*), smooth (*T. fruticans*) or of both types (*T. barbeyanum*). The hairy group, except *T. brevifolium*, is specified with oil glands of either smooth (*T. fruticans*) or reticulate-surfaces (*T. barbeyanum* and *T. campanulatum*). The nutlet sculpture is rugate to ruminate (*T. barbeyanum*), striate to ruminate (*T. brevifolium*) and striate with distinct protrusions (*T. fruticans*). These results are in agreement with Dinç *et al.* (2008) stating the presence or absence of both trichomes and oil glands on the nutlet is among the most useful taxonomic characters in *Teucrium*, and could be used as a taxonomic marker in the infrageneric classification of the genus.

In the glabrous species, the nutlet sculpture is reticulate with three different forms of areoles: (1) deep and uniformly distributed (*T. apollinis*), (2) deep and shallow towards the abscission scar (*T. capitatum*, *T. davaeanum* and *T. polium* subsp. *flavovirens*) and (3) shallow but shallower towards the abscission scar (*T. lini-vaccarii, T.polium* and *T. zanonii*). The reticulate sculpture of *T. capitatum* is discerned by isodiametric cells with convex external periclinal walls, contrary to other species with isodiametric cells with concave external periclinal walls.

According to Myers *et al.* (2000), the exceptional concentrations of endemic species are undergoing exceptional loss of habitat could constitute a kind of "silver bullet" for biodiversity conservation. In particular, the Libyan wild plants are mainly represented by scattered individuals and the decreasing of population size may lead to a genetic drift (Boulila *et al.* 2008). Thus, there is an urgent need for international assistance to collect the endangered plants and to conserve their genetic resources (Al-Idrissi *et al*. 1996). In accordance with 1997 IUCN Red List of Threatened Plants, three species are registered, *T. apollinis, T. barbeyanum* and *T. zanonii*, and this study suggests the inclusion of both *T. davaeanum* and *T. lini-vaccari.*

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